OXFORD

International Journal of Neuropsychopharmacology (2017) 20(12): 1005–1012

doi:10.1093/ijnp/pyx073
Advance Access Publication: August 14, 2017
Regular Research Article

REGULAR RESEARCH ARTICLE

Lack of Ovarian Secretions Reverts the Anabolic Action of Olanzapine in Female Rats

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Abstract

Background: Olanzapine is an orexigenic antipsychotic drug associated with serious metabolic adverse effects in humans. Development of valid rodent models for antipsychotic-induced metabolic adverse effects is hampered by the fact that such effects occur in females only. Estradiol is a predominant female hormone that regulates energy balance. We hypothesized that the female-specific hyperphagia and weight gain induced by olanzapine in the rat are dependent on the presence of estrogens.
Methods: Female sham-operated or ovariectomized rats were treated with a single injection of olanzapine depot formulation. Food intake, body weight, plasma lipids, lipogenic gene expression, energy expenditure, and thermogenic markers including brown adipose tissue uncoupling protein 1 protein levels were measured. Olanzapine was also administered to ovariectomized rats receiving estradiol replacement via the subcutaneous (peripheral) or intracerebroventricular route.
Results: Orexigenic effects of olanzapine were lost in ovariectomized female rats. Ovariectomized rats treated with olanzapine had less pronounced weight gain than expected from their food intake. Accordingly, brown adipose tissue temperature and protein levels of uncoupling protein 1 were elevated. Replacement in ovariectomized rats with either peripherally or centrally

Received: March 14, 2017; **Revised:** July 8, 2017; **Accepted:** August 8, 2017

Significance Statement

Impulsivity and reward sensitiv Olanzapine is a type of medication used to treat symptoms of schizophrenia. Unfortunately, the drug has serious side effects such as increased appetite, weight gain, and diabetes. To find out how olanzapine induces these side effects, it is important to develop animal models. Rats are frequently used in experiments, but unlike in patients, only female rats develop weight gain when treated with olanzapine. The female sex hormone estradiol has an important role in regulation of body weight. We investigated whether the presence of estradiol is required for female rats to gain weight when treated with olanzapine. Indeed, sterilized rats lacking estrogen did not overeat or gain weight when given olanzapine. When estradiol was replaced in sterilized rats, some of the effects of olanzapine were restored. However, the absence or presence of estradiol could not explain all aspects of weight gain caused by olanzapine in our rat model.

administered estradiol reduced food intake and body weight. Cotreatment with olanzapine blocked the anorexigenic effect of peripheral, but not central estradiol.

Conclusions: Our results indicate that the ovarian hormone estradiol plays an important role in olanzapine-induced hyperphagia in female rats and pinpoint the complex effects of olanzapine on the balance between energy intake and thermogenesis.

Keywords: antipsychotics, weight gain, energy expenditure, estradiol

Introduction

Schizophrenia, a debilitating mental disorder with a lifetime prevalence approximating 0.7%, constitutes a significant socio-economic burden globally (McGrath et al., 2008). Antipsychotic agents are indispensable in the treatment of schizophrenia. Unfortunately, some antipsychotic agents, particularly the atypical antipsychotics olanzapine (OLZ) and clozapine, have widely recognized metabolic adverse effects such as weight gain, dyslipidemia, and type 2 diabetes (Leucht et al., 2013). In addition, while antipsychotics provide relief from positive symptoms such as hallucinations and delusions, effects on negative symptoms, such as affective flattening and anhedonia as well as cognitive impairment, are limited (Remington et al., 2016).

The therapeutic effect of antipsychotic drugs is mediated via blockage of dopamine D2 receptors, but other mechanisms may also be of relevance. The molecular underpinnings for both therapeutic and metabolic effects of antipsychotic agents remain elusive. Rodents are frequently used in preclinical experiments designed to shed light on these matters, in particular on drug-induced metabolic disturbances. In the rat, unlike in humans, some aspects of antipsychotic-induced metabolic adverse effects are sex specific (Boyda et al., 2010). In female rats, the effect of OLZ mimics the clinical situation, with hyperphagia, weight gain, increased plasma lipids, and activated transcription of lipid biosynthesis genes after treatment (Albaugh et al., 2006; Vik-Mo et al., 2009; Skrede et al., 2012). In male rats, on the other hand, antipsychotic drug treatment does not lead to weight gain, although OLZ has been shown to increase several plasma lipid species, accompanied by elevated hepatic transcription of lipogenic genes (Ferno et al., 2015).

The lifetime incidence of schizophrenia is 40% higher in males than in females (McGrath et al., 2008), with onset of the disorder peaking significantly later in women (Markham, 2012). Based on these and other data, the ovarian steroids, such as estrogens, have been suggested to contribute to the lower incidence of schizophrenia in females through its neuroprotective properties (Crider and Pillai, 2017). As part of the search for improved pharmacological treatment strategies for schizophrenia, several clinical studies have pointed towards beneficial effects of estrogen/estrogen-modulating agents in female patients suffering from schizophrenia (Heringa et al., 2015). In this context, estradiol (E2), the physiologically most potent estrogen, is highly relevant in the control of energy homeostasis (Mauvais-Jarvis et al., 2013; Lopez and Tena-Sempere, 2015). Decreased E2 levels due to menopause in humans or ovariectomy (OVX) in rodents are associated with hyperphagia, reduced energy expenditure, and weight gain, effects that are reversed by administration of exogenous E2 to OVX rats (Mauvais-Jarvis et al., 2013; Martinez de Morentin et al., 2014, 2015) and hormonal replacement therapy in postmenopausal women (Mauvais-Jarvis et al., 2013).

To address whether physiological E2 milieu is required in the rat model of antipsychotic-induced weight gain, we first administered a depot injection of OLZ to OVX female rats. We then explored how E2 replacement at physiological levels (Martinez de Morentin et al., 2015) affected the OLZ-induced effects. Our data show that OLZ treatment leads to hyperphagia and weight gain in sham-operated (gonadal-intact), but not in OVX rats. The s.c. E2 replacement in OVX rats seems to partially rescue the OLZ-induced hyperphagia, but this partial reversion was not observed when E2 replacement was central. Our findings support the suggestion that E2 is a relevant hormone for the orexigenic effects of OLZ in female rats.

Experimental Procedures

Animals

All experiments were approved by the USC Ethical Committee (project license 15010/14/006). Adult female Sprague-Dawley rats (2–3 months old) were kept under standard conditions with an artificial 12-hour-light/-dark cycle (lights on 8:00 AM) and constant 48% humidity. Animals were housed individually and allowed access to tap water and free (ad libitum) access to

standard laboratory chow (Special Diets Services) during the experimental period.

Surgical Procedures

Bilateral OVX or sham surgery was performed on female Sprague-Dawley rats under ketamine/xylacine anesthesia (50 mg/kg i.p.) as previously described (Martinez de Morentin et al., 2014, 2015). All treatment schemes in OVX rats were started 2 weeks after surgery to ensure a total washout of endogenous ovarian hormones (Martinez de Morentin et al., 2014, 2015). In the intracerebroventricular (ICV) experiment, ICV cannulas were surgically implanted as previously reported (Lopez et al., 2004). Animals were left to recover for 2 days before the initiation of treatment (see below).

Treatment Schedules

Two weeks after sham surgery or OVX, rats (n = 9 in each treatment group) received one intramuscular injection of either OLZ pamoate depot formulation (100 mg/kg; ZypAdhera, Eli Lilly) or vehicle (VEH) solution (injection volume 160 μL/250 g body weight) (Skrede et al., 2014). A subgroup of the OVX rats (n = 8) received daily s.c. injections of E2 (E2 benzoate; standard dose of 2 μg dissolved in 100 μL of sesame oil; both from Sigma) or vehicle (100 μL of sesame oil; control rats). In the ICV experiment, vehicle (5 μL of saline containing 10% of dimethyl sulfoxide) or E2 (17β-estradiol, 5 nmol dissolved in 5 μL of vehicle) was administered through the inserted cannulae at 8 AM and 8 PM for 7 days (n = 9–10 in each treatment group). At 24 hours after the first ICV E2 dose, animals received a single intramuscular dose of OLZ pamoate 100 mg/kg. Animals and chow were weighed daily. Rats treated with SC E2 were sacrificed after 8 days of treatment, that is, on day 22 after surgical procedures (OVX or sham). Tissue and blood samples were harvested and stored at -80°C until further analysis. Due to limited durability of ICV cannulas, rats treated with ICV E2 were killed after 6 days of treatment, that is, on day 20 after OVX or sham surgery.

Calorimetric System and Nuclear Magnetic Resonance

In a separate experiment, OVX rats (n = 6 in each treatment group) received an intramuscular injection of either OLZ pamoate depot formulation (100 mg/kg) or VEH. During the first 6 days after injection, energy expenditure (EE), food intake, body weight, and locomotor activity (LA) were measured using a calorimetric system (LabMaster, TSE Systems). Animals were placed in a temperature-controlled (24°C) box through which air was pumped. After calibrating the system with the reference gases (20.9% O_2, 0.05% CO_2, and 79.05% N_2), the metabolic rate was measured as previously shown (Imbernon et al., 2013; Martinez de Morentin et al., 2014). EE, respiratory quotient (VCO_2/VO_2), food intake (FI), and LA were recorded every 30 minutes. Animals were placed for adaptation for 1 week before starting the measurements. To adjust for body composition, lean mass was measured using nuclear magnetic resonance (Whole Body Composition Analyzer, EchoMRI). EchoMRI is a body composition analyzer for live subjects measuring body fat and lean masses with short scan times to ensure the comfort of the animals. Animals do not need to be anesthetized or subjected to other special preparation before measurement. They are placed in a holder of custom-defined size during the measurement (measuring time: 0.5–3.2 minutes). Two measurements were done for each animal, as previously shown (Imbernon et al., 2013; Martinez de Morentin et al., 2014).

RNA Extraction, cDNA Synthesis, and Real-Time PCR

RNA extraction, cDNA synthesis, and real-time PCR were performed as previously described (Ferno et al., 2005; Martinez de Morentin et al., 2014, 2015). Relative gene expression levels were determined using the comparative ΔCt method using β-actin (Actb) and ribosomal protein, large, (Rplp0) as endogenous controls. For primer sequences, see Table 1.

Western Blotting

Tissue homogenization and western blotting were performed as previously described (Martinez de Morentin et al., 2014, 2015). The primary antibodies used were: Uncoupling protein 1 (UCP1) (ab10983; RRID: AB_2241462, polyclonal, lot #GR249119-1, Abcam; dilution 1:10 000) and α-tubulin (T5168; RRID: AB_477579, mouse clone B-5-1-2, ascites fluid. Lot#035M4878V, Sigma; dilution 1:5000). Signal intensity measurements were performed using the ImageJ software (National Institutes of Health).

Biochemical Parameters

Glucose and lipids (total cholesterol [tot-CHOL], free cholesterol, HDL cholesterol [HDL-CHOL], LDL cholesterol [LDL-CHOL], free fatty acids [FFA], phospholipids [PLIP], and triglycerides [TG]) were measured enzymatically in plasma samples obtained from fasted rats using the Hitachi 917 system (Roche Diagnostics) as previously described (Jassim et al., 2012).

Statistical Analysis

Food intake and weight gain were analyzed using 2-way ANOVA repeated measures. For all other comparisons, we used 2-sided Student's *t* test. $P \leq .05$ was considered statistically significant. PASW Statistics Version 18 (PASW Statistics, IBM) was used for all calculations. Data are presented as mean ± SEM.

Table 1. Primer Sequences Used for Real-Time PCR

Gene	Main function	Accession number	Fwd primer	Rev primer
Fatty acid synthase (*Fasn*)	Fatty acid synthesis	NM_017332	CCATCATCCCCTTGATGAAGA	GTTGATGTCGATGCCTGTGAG
Stearoyl-CoA 9-desaturase; stearoyl-CoA desaturase 1 (*Scd1*)	Fatty acid desaturation	NM_139192	TCAATCTCGGGAGAACATCC	CATGCAGTCGATGAAGAACG
β-Actin (*Actb*)	Endogenous control	NM_031144	TACAGCTTCACCACCACAGC	CTTCTCCAGGGAGGAAGAGG
Rattus norvegicus ribosomal protein lateral stalk subunit P0 (*Rplp0*)	Endogenous control	NM_022402.2	CATTGAAATCCTGAGCGATGT	AGATGTTCAACATGTTCAGCAG

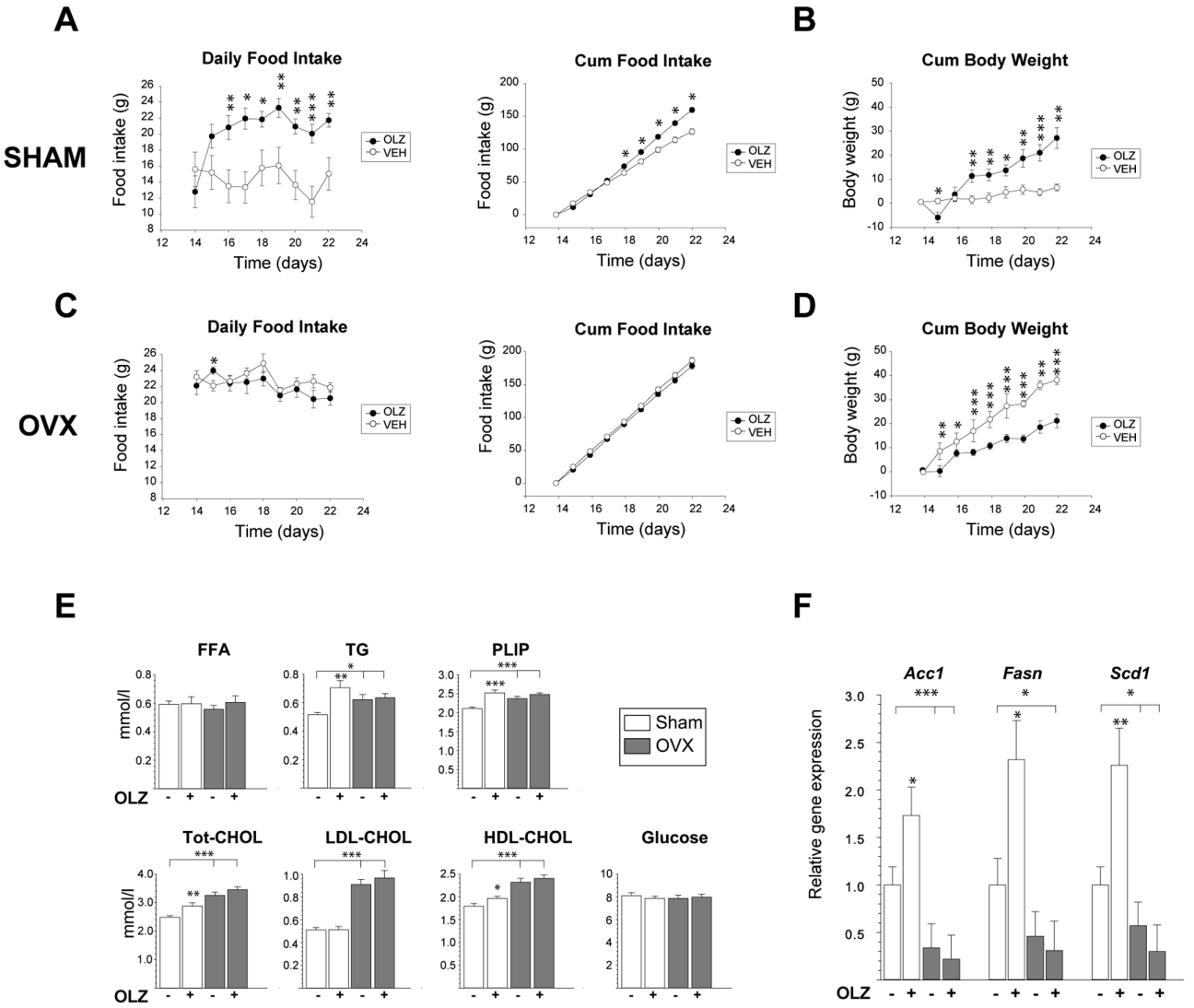

Figure 1. (A) Daily and cumulative food intake and (B) cumulative body weight gain in female sham operated rats treated with single depot-injections of either vehicle (VEH) or olanzapine (OLZ, 100 mg/kg) for 8 days, initiated 14 days after surgery. (C) Daily and cumulative food intake and (D) cumulative body weight gain in ovariectomized (OVX) rats treated with VEH or OLZ for 8 days, initiated 14 days after OVX. *P ≤ .05, **P ≤ .01, ***P ≤ .001, OLZ compared with VEH. (E) Plasma lipid levels in female sham (white bars) or OVX (grey bars) rats treated with single depot injections of VEH or OLZ (100 mg/kg) for 8 days. FFA, free fatty acids; GLUC, glucose; HDL-CHOL, HDL cholesterol; LDL-CHOL, LDL cholesterol; PLIP, phospholipids; TG, triglycerides; Tot-CHOL, total cholesterol. *P ≤ .05, **P ≤ .01, ***P ≤ .001, compared with sham VEH. (F) Transcription levels of key lipogenic genes in gonadal white adipose tissue from female sham (white bars) or OVX (grey bars) rats treated with single depot injections of VEH or OLZ (100 mg/kg) for 8 days. *Acc1*, acetyl-CoA carboxylase 1; *Fasn*, fatty acid synthase; *Scd1*, stearoyl-CoA desaturase 1; *P ≤ .05, **P ≤ .01, ***P ≤ .001, OLZ compared with sham VEH.

Results

OLZ Reduces Weight Gain in OVX Female Rats

As expected, OLZ treatment in sham-operated female rats resulted in significant increases in both daily and cumulative food intake (Figure 1a) as well as body weight gain (Figure 1b). Also in agreement with our previously published data (Martinez de Morentin et al., 2014, 2015), OVX resulted in marked hyperphagia (Figure 1c) and elevated weight gain (Figure 1d) to levels that were in the range of the OLZ-induced effects in gonadal-intact rats. OLZ administration to OVX rats did not stimulate food intake further, and interestingly, body weight gain was in fact reduced by OLZ relative to VEH-treated rats (Figure 1d).

OLZ Does Not Impact Plasma Lipids in OVX Female Rats

In sham-operated female rats (Figure 1e, white bars), OLZ treatment caused significant increases in plasma TGs, PLIPs, Tot-CHOL, and HDL-CHOL, but not in LDL-CHOL and FFAs. In line with the effect of OVX on food intake and body weight gain, all plasma lipid parameters were higher in OVX vs sham-operated rats, with the exception of FFA, where levels remained unaltered (Figure 1e). In OVX rats (Figure 1b, grey bars), OLZ did not further increase plasma lipid levels. OLZ had no effect on fasting glucose levels in either OVX or sham-operated rats (Figure 1e).

OLZ Does Not Impact Adipose Tissue Lipogenic Gene Expression in OVX Female Rats

As expected (Skrede et al., 2012), in gonadal white adipose tissue from gonadal-intact female rats, OLZ induced a significant upregulation of genes involved in fatty acid biosynthesis, such as acetyl-CoA carboxylase 1, fatty acid synthase, and stearoyl-CoA desaturase 1 (Figure 1f, white bars). In OVX rats (Figure 1f, grey bars), the expression levels of all these genes were markedly lower than in sham rats, and notably, OLZ failed to induce significant transcriptional changes for any of the genes in the absence of ovarian secretions.

Peripheral E2 Replacement Partially Recovers OLZ-Induced Orexigenic Effect in OVX Rats

To investigate whether estrogen replacement in OVX rats could restore the orexigenic action of OLZ, OVX rats received daily s.c. injections of E2 while co-administered with either OLZ or VEH as single intramuscular depot injections. As expected, E2 decreased food intake and body weight gain in OVX rats (Figure 2a; compare open circles with open triangles). However, when E2 rats were co-treated with OLZ (Figure 2a), an interaction effect was observed, with daily and cumulative food intake significantly higher in E2+OLZ than in E2+VEH rats and body weight displaying a slight trend towards elevation (Figure 2b). On the contrary, in OVX rats without E2 (Figure 2b), OLZ significantly reduced body weight vs VEH-treated OVX rats, which occurred independent of changes in food intake.

OLZ Increases UCP1 Expression in BAT

The fact that OLZ decreased body weight gain in OVX female rats without concomitant reduction in food intake suggested that OLZ stimulates EE in this setting. We therefore measured UCP1 protein levels in BAT. In agreement with the phenotype, we found increased levels of BAT UCP1 in OVX female rats treated with OLZ relative to VEH (Figure 2c). Treatment of OVX female rats with E2 did not change this effect, as UCP1 levels were still increased to a similar magnitude in OVX rats cotreated with E2+OLZ vs rats treated with VEH+E2 (Figure 2c). Of note, BAT UCP1 levels were elevated by OLZ also in sham rats (Figure 2c), which occurred despite the significantly increased body weight induced by OLZ in this setting (Figure 1b). However, food intake was markedly stimulated by OLZ in these sham rats (Figure 1a), potentially compensating for any weight loss caused by increased EE.

Central E2 Replacement in OVX Rats Does Not Recover OLZ-Induced Orexigenic Effect

To address whether the observed interactive effect of E2 and OLZ on food intake was centrally mediated, the effect of OLZ in OVX rats treated ICV with E2 was investigated. As expected, ICV E2 markedly reduced the hyperphagia and weight gain in OVX rats relative to ICV VEH (Figure 3a-b). Surprisingly, however, an intramuscular injection of OLZ did not counteract the anorexigenic effect of E2 in this setting, with no significant difference between ICV E2 and ICV E2+OLZ on either food intake or body weight (Figure 3a-b). In line with these data, we observed a significant increase in BAT temperature in all animals treated with E2 (Figure 3c), whereas there was no difference in BAT temperature between ICV E2 and ICV E2+OLZ (Figure 3c). In an additional experiment, we investigated the isolated effect of an OLZ on BAT temperature in OVX rats, without ICV E2 cotreatment. In agreement with the observed OLZ-induced increase in BAT UCP1 levels (Figure 2c), an intramuscular injection of OLZ significantly increased BAT temperature (Figure 3d). This effect occurred without any significant changes in food intake (OLZ: 20.9 ± 2.5 g/d vs VEH: 17.5 ± 1.3) or body weight (OLZ: -8.0 ± 8.6 g vs VEH: -5.3 ± 7.8 g). Of note, during this short observation period of 3 days, total EE or the respiratory quotient were not significantly elevated by the OLZ treatment (supplementary Figure S1a-b). This may, at least in part, be explained by the concomitant reduced LA (supplementary Figure S1d-e), which was expected from the sedative effect of OLZ.

Discussion

Conditions of suppressed or lacking ovarian estrogen secretions are associated with a positive energy balance (Mauvais-Jarvis et al., 2013; Lopez and Tena-Sempere, 2015). Hence, in the OVX rats we found the expected elevation of food intake, body weight, and plasma lipids. Interestingly, treatment with OLZ yielded strikingly different results in ovarian-intact and OVX rats. In sham rats, OLZ led to elevation of food intake, body weight, plasma lipids, and lipogenic gene expression in white adipose tissue, as previously shown (Ferno et al., 2011; McNamara et al., 2011; Skrede et al., 2012). In contrast, OLZ had no effect on food intake, plasma lipids, or lipogenic gene expression in OVX rats in which body weight gain was actually reduced by OLZ.

A possible explanation for the lack of orexigenic and dysmetabolic effects of OLZ in OVX rats is that the anabolic effects of OLZ were masked by OVX-induced hyperphagia and elevated plasma

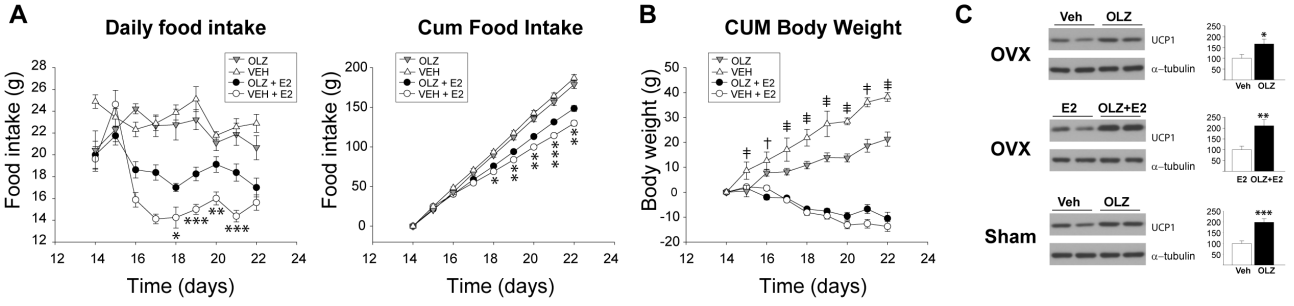

Figure 2. (A) Daily and cumulative food intake and (B) cumulative body weight gain in female ovariectomized (OVX) rats treated with single depot injections of olanzapine (OLZ) (100 mg/kg) or vehicle (VEH), with (open circles) or without (triangles) cotreatment with daily s.c. injections of vehicle or E2 (2 μg/d) for 8 days. *P ≤ .05, **P ≤ .01, ***P ≤ .001, E2+OLZ compared with E2+VEH; +P ≤ .05, ǂP ≤ .01, ǂǂP ≤ .001, OLZ compared with VEH. (C) Uncoupling protein 1 (UCP1) western blots in brown adipose tissue (BAT) from female sham or OVX rats treated with a single injection of VEH, OLZ (100 mg/kg), or OLZ+E2 (2 μg) for 8 days. *P ≤ .05, **P ≤ .01, OLZ compared with VEH and OLZ+E2 compared with VEH+E2.

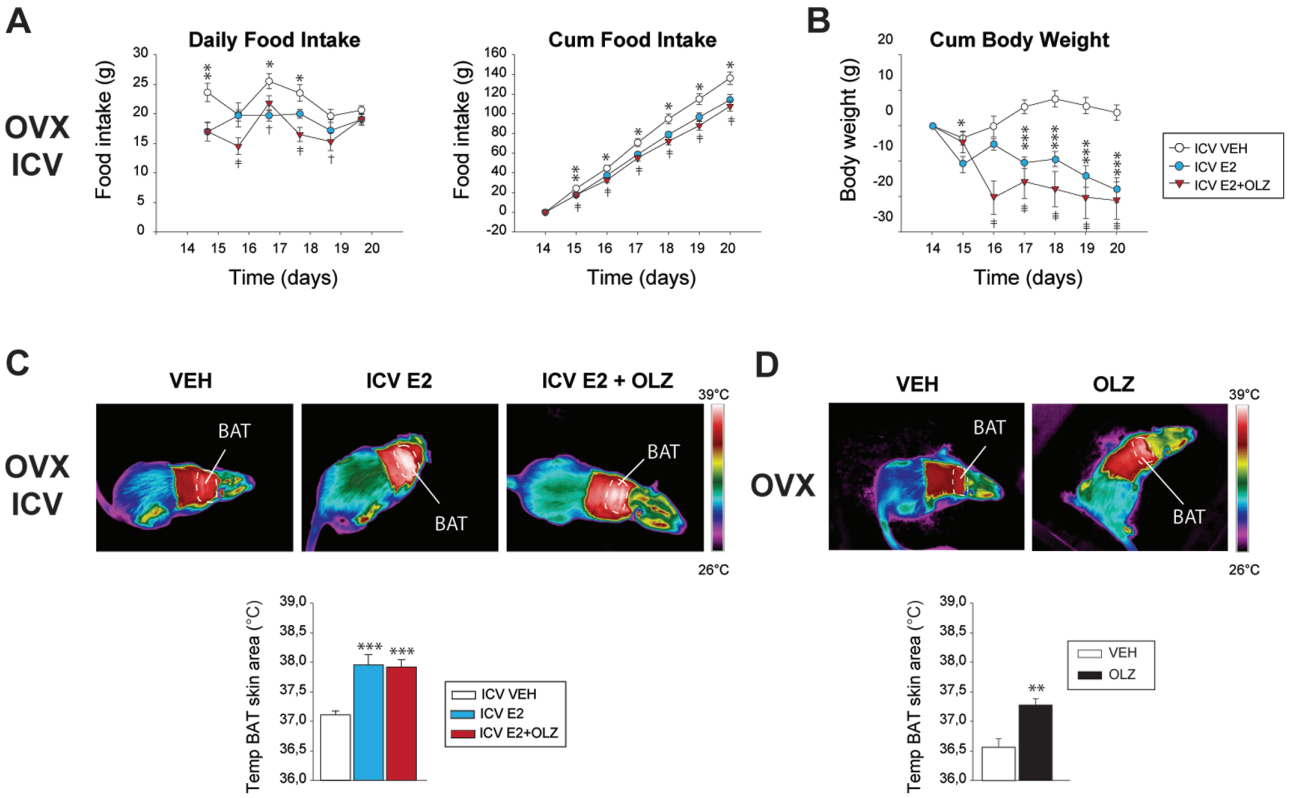

Figure 3. (A) Daily and cumulative food intake and (B) cumulative body weight gain in female ovariectomized (OVX) rats treated with single depot injections of olanzapine (OLZ) (100 mg/kg) or vehicle (VEH), with cotreatment with daily replacement with intracerebroventricular (ICV) VEH or E2. *P ≤ .05, **P ≤ .01, ***P ≤ .001, ICV E2 compared with ICV VEH. ǂP ≤ .01, ǂǂP ≤ .001, ICV E2+OLZ compared with ICV E2+VEH. (C) Infrared thermal images and quantification of temperature of BAT skin area of in female OVX rats treated with single depot injections of OLZ (100 mg/kg) or VEH, with cotreatment with daily replacement with ICV VEH or E2. Images were taken after 4 days of E2 IVC and 3 days of OLZ initiation, respectively. ***P ≤ .001, ICV E2+VEH or ICV E2+OLZ compared with ICV VEH. (D) Infrared thermal images and quantification of temperature of BAT skin area of female OVX rats treated with single depot injections of OLZ (100 mg/kg) or VEH. Images were taken 3 days after the initiation of treatment. **P ≤ .01, ICV E2+VEH or ICV E2+OLZ compared with ICV VEH.

lipids. However, the fact that lipogenic gene expression levels were lower in OVX than in sham rats argues against the validity of this explanation, at least with regard to the whole set of metabolic alterations of OLZ. Notably, the effect of OLZ in OVX rats has been examined previously, but contrary to our findings, OLZ treatment was reported to induce hyperphagia and weight gain in OVX rats (Park et al., 2010). However, the previous study was carried out on a diabetic background (90% pancreatectomy) in rats fed a high-fat diet, whereas here we used nondiabetic Sprague-Dawley rats fed standard chow. Furthermore, in our study, OLZ was administered as a depot formulation, which yields high and stable OLZ serum concentrations (Skrede et al., 2014).

The observation that OLZ reduced body weight in OVX female rats without concomitant changes in food intake suggests that in the absence of ovarian secretions OLZ stimulates thermogenesis. While findings of increased BAT temperature and UCP1 protein levels supported this theory, net EE was unaltered in OVX rats treated with OLZ, possibly due to sedation and subsequent reduced LA. Interestingly, BAT UCP1 levels were also elevated by OLZ in sham-operated rats despite that both food intake and weight gain were increased by the drug in this situation. Thus, the marked hyperphagia observed in sham-operated rats treated with OLZ seems to overshadow the increase in thermogenesis, with a net increase in body weight as the observed phenotype. In OVX rats, OLZ does not induce hyperphagia but increases thermogenesis, as demonstrated by the augmentation of BAT temperature and UCP1 protein levels, potentially explaining the lack of body weight gain.

We further examined the effect of E2 substitution on the OLZ-induced phenotype. Daily injections of SC or ICV E2 to OVX female rats led to reversal of hyperphagia and weight gain, as expected (Martinez de Morentin et al., 2014, 2015). This anorexigenic effect was blunted by OLZ in rats receiving peripheral, but not central, E2. OVX rats treated with peripheral E2 and OLZ displayed markedly higher food intake than OLZ-treated OVX rats without E2 replacement. These data show that when OVX rats are substituted with E2 via the peripheral route, OLZ regains its orexigenic potential. However, these data should be interpreted with caution, since the orexigenic effect of OLZ during cotreatment with E2 may be a result of its action as an inhibitor of E2s' anorexigenic effect rather than a direct stimulator of food intake. Of note, the elevated food intake did not translate into significantly higher body weight gain in the group of OVX rats treated s.c. with E2 and OLZ. In keeping with this observation and the results discussed above, BAT UCP1 levels were elevated by OLZ also when cotreated with E2. In rats receiving central E2, we also showed that BAT temperature was increased, again indicative of increased thermogenesis. Nevertheless, OLZ did not further affect BAT temperature in these rats.

Taken together, the findings support our hypothesis that the presence of estradiol is important for the hyperphagia and weight gain associated with the antipsychotic agent OLZ in the rat. Notably, the thermogenic effects of OLZ seem to occur independent of estradiol.

The molecular underpinnings of our results should be further evaluated in future studies. OLZ is believed to mediate its

orexigenic effects via its antagonistic effects on serotonin 5HT2C and histamine H1 receptors (Reynolds and Kirk, 2010). We have previously demonstrated that OLZ-induced hyperphagia is associated with upregulation of the orexigenic neuropeptides NPY and AgRP and downregulation of the anorexigenic neuropeptide POMC in the hypothalamus (Ferno et al., 2011). Furthermore, we and others have suggested that activation of AMPK in the arcuate nucleus of the hypothalamus is important (Kim et al., 2007; He et al., 2014; Skrede et al., 2014). The impact of OLZ treatment on AMPK activation and neuropeptide levels in OVX rats could be a topic of targeted investigations. Considering that NPY is likely to mediate OVX-induced hyperphagia, this neuropeptide would be of particular interest to investigate the E2 OLZ interaction effect.

Direct translation of our rodent data should be done with caution. While some clinical trials have documented that female gender is a risk factor for antipsychotic-induced weight gain, gender differences seem to be far less pronounced in patients than in rodents. It must be stressed, though, that gender aspects are often overlooked in clinical trials; this issue requires more attention through targeted clinical studies. Furthermore, the involvement of EE in antipsychotic-induced weight gain remains under-investigated in humans. It is possible that OLZ stimulates EE also in humans, and the balance between orexigenic and energy dissipation effects may contribute to the large inter-individual variation observed among patients during treatment with antipsychotic agents. Correspondingly, studies in healthy volunteers and patients treated with antipsychotic agents have yielded conflicting results with regard to resting EE (Cuerda et al., 2014). All in all, further research is warranted to determine the role of EE in antipsychotic-induced metabolic dysfunction.

As part of the search for improved pharmacological strategies to treat schizophrenia, several clinical studies have pointed toward beneficial effects of treatment with estrogen/estrogen-modulating agents in female patients suffering from schizophrenia (Heringa et al., 2015). The present study documents that in the rat, key features of the metabolic impact of OLZ are dependent on ovarian secretion. The results highlight important aspects that need to be considered in clinical trials, both involving antipsychotic monotherapy and add-on treatment with estrogen and its related pharmacological agents.

Acknowledgments

The authors thank Marianne S. Nævdal and Liv Kristine Øysæd for excellent technical assistance.

Funding

This work was supported by the Research Council of Norway (V.M.S.: CoE grant to Norwegian Centre for Mental Disorders Research; project number 223273), Stiftelsen Kristian Gerhard Jebsen (V.M.S.), Dr. Einar Martens Foundation (V.M.S., S.S., J.F.), Helse Vest RHF (J.F. and G.M.: the Western Norway Regional Health Authority), the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement no. 281854,: the ObERStress European Research Council Project (M.L.), Junta de Andalucía (M.T.-S.: P12-FQM-01943), Xunta de Galicia (R.N.: 2015-CP080 and 2016-PG057; M.L.: 2015-CP079), Instituto de Salud Carlos III and Fondo Europeo de Desarrollo Regional (ISCIII, FEDER; M.L.: PIE13/00024), MINECO co-funded by the FEDER Program of EU (R.N.: BFU2015-70664; M.T.S.: BFU2014-2502157581-P; M.L.: SAF2015-71026-R and BFU2015-70454-REDT/Adipoplast). I.G.-G. is a recipient of a fellowship from Ministerio de Educación, Cultura y Deporte (FPU12/01827).

Statement of Interest

None.

References

Albaugh VL, Henry CR, Bello NT, Hajnal A, Lynch SL, Halle B, Lynch CJ (2006) Hormonal and metabolic effects of olanzapine and clozapine related to body weight in rodents. Obesity (Silver Spring) 14:36–51.

Boyda HN, Tse L, Procyshyn RM, Honer WG, Barr AM (2010) Preclinical models of antipsychotic drug-induced metabolic side effects. Trends Pharmacol Sci 31:484–497.

Crider A, Pillai A (2017) Estrogen signaling as a therapeutic target in neurodevelopmental disorders. J Pharmacol Exp Ther 360:48–58.

Cuerda C, Velasco C, Merchan-Naranjo J, Garcia-Peris P, Arango C (2014) The effects of second-generation antipsychotics on food intake, resting energy expenditure and physical activity. Eur J Clin Nutr 68:146–152.

Ferno J, Ersland KM, Duu IH, Gonzalez-Garcia I, Fossan KO, Berge RK, Steen VM, Skrede S (2015) Olanzapine depot exposure in male rats: dose-dependent lipogenic effects without concomitant weight gain. Eur Neuropsychopharmacol 25:923–932.

Ferno J, Raeder MB, Vik-Mo AO, Skrede S, Glambek M, Tronstad KJ, Breilid H, Lovlie R, Berge RK, Stansberg C, Steen VM (2005) Antipsychotic drugs activate SREBP-regulated expression of lipid biosynthetic genes in cultured human glioma cells: a novel mechanism of action? Pharmacogenomics J 5:298–304.

Ferno J, Varela L, Skrede S, Vazquez MJ, Nogueiras R, Dieguez C, Vidal-Puig A, Steen VM, Lopez M (2011) Olanzapine-induced hyperphagia and weight gain associate with orexigenic hypothalamic neuropeptide signaling without concomitant AMPK phosphorylation. PLoS One 6:e20571.

He M, Zhang Q, Deng C, Wang H, Lian J, Huang XF (2014) Hypothalamic histamine H1 receptor-AMPK signaling time-dependently mediates olanzapine-induced hyperphagia and weight gain in female rats. Psychoneuroendocrinology 42:153–164.

Heringa SM, Begemann MJ, Goverde AJ, Sommer IE (2015) Sex hormones and oxytocin augmentation strategies in schizophrenia: a quantitative review. Schizophr Res 168:603–613.

Imbernon M, Beiroa D, Vazquez MJ, Morgan DA, Veyrat-Durebex C, Porteiro B, Diaz-Arteaga A, Senra A, Busquets S, Velasquez DA, Al-Massadi O, Varela L, Gandara M, Lopez-Soriano FJ, Gallego R, Seoane LM, Argiles JM, Lopez M, Davis RJ, Sabio G, Rohner-Jeanrenaud F, Rahmouni K, Dieguez C, Nogueiras R (2013) Central melanin-concentrating hormone influences liver and adipose metabolism via specific hypothalamic nuclei and efferent autonomic/JNK1 pathways. Gastroenterology 144:636–649 e636.

Kim SF, Huang AS, Snowman AM, Teuscher C, Snyder SH (2007) From the cover: antipsychotic drug-induced weight gain mediated by histamine H1 receptor-linked activation of hypothalamic AMP-kinase. Proc Natl Acad Sci USA 104:3456–3459.

Leucht S, Cipriani A, Spineli L, Mavridis D, Orey D, Richter F, Samara M, Barbui C, Engel RR, Geddes JR, Kissling W, Stapf MP, Lassig B, Salanti G, Davis JM (2013) Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. Lancet 382:951–962.

Lopez M, Seoane LM, Tovar S, Nogueiras R, Dieguez C, Senaris R (2004) Orexin-A regulates growth hormone-releasing hormone mRNA content in a nucleus-specific manner and somatostatin mRNA content in a growth hormone-dependent fashion in the rat hypothalamus. Eur J Neurosci 19:2080–2088.

Lopez M, Tena-Sempere M (2015) Estrogens and the control of energy homeostasis: a brain perspective. Trends Endocrinol Metab 26:411–421.

Markham JA (2012) Sex steroids and schizophrenia. Rev Endocr Metab Disord 13:187–207.

Martinez de Morentin PB, Gonzalez-Garcia I, Martins L, Lage R, Fernandez-Mallo D, Martinez-Sanchez N, Ruiz-Pino F, Liu J, Morgan DA, Pinilla L, Gallego R, Saha AK, Kalsbeek A, Fliers E, Bisschop PH, Dieguez C, Nogueiras R, Rahmouni K, Tena-Sempere M, Lopez M (2014) Estradiol regulates brown adipose tissue thermogenesis via hypothalamic AMPK. Cell Metab 20:41–53.

Martinez de Morentin PB, Lage R, Gonzalez-Garcia I, Ruiz-Pino F, Martins L, Fernandez-Mallo D, Gallego R, Ferno J, Senaris R, Saha AK, Tovar S, Dieguez C, Nogueiras R, Tena-Sempere M, Lopez M (2015) Pregnancy induces resistance to the anorectic effect of hypothalamic malonyl-CoA and the thermogenic effect of hypothalamic AMPK inhibition in female rats. Endocrinology 156:947–960.

Mauvais-Jarvis F, Clegg DJ, Hevener AL (2013) The role of estrogens in control of energy balance and glucose homeostasis. Endocr Rev 34:309–338.

McGrath J, Saha S, Chant D, Welham J (2008) Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev 30:67–76.

McNamara RK, Jandacek R, Rider T, Tso P, Cole-Strauss A, Lipton JW (2011) Atypical antipsychotic medications increase postprandial triglyceride and glucose levels in male rats: relationship with stearoyl-CoA desaturase activity. Schizophr Res 129:66–73.

Park S, Hong SM, Ahn IL, Kim DS, Kim SH (2010) Estrogen replacement reverses olanzapine-induced weight gain and hepatic insulin resistance in ovariectomized diabetic rats. Neuropsychobiology 61:148–161.

Remington G, Foussias G, Fervaha G, Agid O, Takeuchi H, Lee J, Hahn M (2016) Treating negative symptoms in schizophrenia: an update. Curr Treat Options Psychiatry 3:133–150.

Reynolds GP, Kirk SL (2010) Metabolic side effects of antipsychotic drug treatment--pharmacological mechanisms. Pharmacol Ther 125:169–179.

Skrede S, Ferno J, Vazquez MJ, Fjaer S, Pavlin T, Lunder N, Vidal-Puig A, Dieguez C, Berge RK, Lopez M, Steen VM (2012) Olanzapine, but not aripiprazole, weight-independently elevates serum triglycerides and activates lipogenic gene expression in female rats. Int J Neuropsychopharmacol 15:163–179.

Skrede S, Martins L, Berge RK, Steen VM, Lopez M, Ferno J (2014) Olanzapine depot formulation in rat: a step forward in modelling antipsychotic-induced metabolic adverse effects. Int J Neuropsychopharmacol 17:91–104.

Vik-Mo AO, Ferno J, Skrede S, Steen VM (2009) Psychotropic drugs up-regulate the expression of cholesterol transport proteins including ApoE in cultured human CNS- and liver cells. BMC Pharmacol 9:10.